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Short communication

Simple and rapid determination of the herbicides glyphosate and glufosinate in river water, soil and carrot samples by gas chromatography with flame photometric detection

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Abstract

A rapid, selective and sensitive gas chromatographic (GC) method for the simultaneous determination of the phosphorus-containing amino acid-type herbicides glyphosate (GLYP), its metabolite aminomethylphosphonic acid (AMPA) and glufosinate (GLUF) in environmental and food samples was developed. After extraction of the sample with water or sodium hydroxide solution, these compounds were converted into their N-isopropoxycarbonyl methyl ester derivatives and then measured by GC using a DB-1701 capillary column with flame photometric detection (FPD). The derivative preparation and GC analysis were accomplished within 20 min. The derivatives were sufficiently volatile and stable, and the FPD response was excellent. The detection limits of AMPA, GLYP and GLUF, at a signal-to-noise ratio of 3, were ca. 8, 12 and 20 pg injected, respectively. The calibration graphs for these compounds in the range 5–200 ng were linear and sufficiently reproducible for quantitative determination. This method could be successfully applied to river water, soil and carrot samples without a preliminary clean-up procedure, and AMPA, GLYP and GLUF could be measured without any interference from co-existing substances. The recoveries of these compounds in these samples were 91–106%.

Keywords: Environmental analysis; Food analysis; Carrot; Soil; Water analysis; Derivatization, GC; Pesticides; Glyphosate; Glufosinate; Aminomethylphosphonic acid; Aminobutylphosphonic acid

1. Introduction

The phosphorus-containing amino acid-type herbicides glyphosate [N-(phosphonomethyl) glycine] (GLYP) and glufosinate [DL-homoalanine-4-yl(methyl)phosphinic acid] (GLUF) have a broad spectrum and are non-selective weedkillers. The herbicidal effect of GLYP is to interrupt aromatic amino acid biosynthesis in plants by inhibition of the enzymes 5-enolpyruvylshikimate-3-phosphate synthase [1,2] or 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase [3]. On the other hand, GLUF, which has become increasingly popular and is also an active metabolite of the tripeptide-type herbicide bialaphos [L-2-amino-4-(hydroxy)(methyl)phosphinyl]butyryl-L-alanine, is to inhibit competitively the enzyme glutamine synthetase [4]. These herbicides are of comparatively low toxicity to animals and humans, and have therefore

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been widely used in agriculture for controlling many annual and perennial weeds, but their effects on non-target organisms and overall environmental fate have not been fully evaluated.

The determination of GLYP and GLUF has been carried out by gas chromatography (GC) based on the preparation of N-heptafluorobutyrylchloroethyl ester [5], N-trifluoroacetyltrifluoroalkyl ester [6–8], N-trifluoroacetylheptafluorobutyl ester [9] and *tert.*-butyldimethylsilyl [10,11] derivatives and high-performance liquid chromatography (HPLC) with both precolumn [12–18] and postcolumn [19–21] derivatization. The GC method could take advantage of the specificity of flame photometric and mass-selective detectors or the extreme sensitivity of an electron-capture detector, but the preparation of the derivatives requires a time-consuming procedure under anhydrous conditions. On the other hand, some of the HPLC methods are highly sensitive with fluorogenic labelling, but they lack specificity and usually require a laborious clean-up procedure such as ion-exchange column chromatography, which may result in some sample loss and lower reproducibility.

Previously, we have developed a convenient and reliable method for the determination of GLYP and its major metabolite aminomethylphosphonic acid (AMPA) as their N-isobutoxycarbonyl (N-isoBOC) methyl ester derivatives by GC with flame photometric detection (FPD) [22]. In this work, we investigated a rapid, selective and sensitive method for the simultaneous determination of AMPA, GLYP and GLUF in water, soil and food samples as their N-isopropoxycarbonyl (isoPOC) methyl ester derivatives by GC–FPD.

2. Experimental

2.1. Reagents

GLYP, AMPA and 4-aminobutylphosphonic acid (ABP) as an internal standard (I.S.) were purchased from Sigma (St. Louis, MO, USA), GLUF from Hoechst Japan (Tokyo, Japan) and bialaphos from Meiji Seika (Tokyo, Japan). Each compound was dissolved in distilled water to make a stock solution at a concentration of 0.1 mg/ml. Isopropyl chloroformate (isoPCF) was obtained from Wako (Osaka, Japan) and used without further purification. N-Methyl-N-nitroso-*p*-toluenesulphonamide and diethylene glycol monomethyl ether for the generation of diazomethane [23] were obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical-reagent grade.

2.2. Preparation of samples

River water was collected from some rivers in Okayama, filtered through filter-paper (Toyo No. 5A), if necessary, and used directly for analysis. Clay loam soils were purchased from a garden shop. After adding 0.5 ml of 0.5 μg/ml ABP (I.S.) to 0.5 g of soil, the sample was extracted three times with 3 ml of 0.2 *M* sodium hydroxide by shaking for 5 min, and then centrifuged at 2000 *g* for 5 min. The combined supernatants were made up to 10 ml with distilled water and used for the analysis. Carrots were purchased from a local retail market. After adding 0.5 ml of 0.5 μg/ml ABP (I.S.) to 0.5 g of carrot, the sample was homogenized in 3 ml of water with a Model LK-21 ultra-disperser (Yamato Kagaku, Tokyo, Japan), centrifuged at 2000 *g* for 5 min and then the precipitate was re-extracted twice with 3 ml of distilled water. The combined supernatants were made up to 10 ml with distilled water and used for analysis. For fortification analysis, to each sample was added AMPA, GLYP and GLUF at the 0.01–1 μg/ml (or μg/ml) level before standing at room temperature overnight. The fortified samples were treated by the method described above.

2.3. Derivatization procedure

To the standard solution containing 5–200 ng of AMPA, GLYP and GLUF or 0.5–2 ml of the sample prepared by the above method were added 0.1 ml of 0.5 μg/ml ABP (I.S.), if necessary, and the solution was adjusted to pH $>$10 with 2 *M* sodium hydroxide. Immediately after

adding 0.05 ml of isoPCF, the solution was vigorously mixed for 10 s with a vortex-type mixer. The reaction mixture was acidified to pH 1–2 with 2 *M* hydrochloric acid and extracted with 3 ml of diethyl ether in order to remove the excess reagent, the ethereal extracts being discarded. The aqueous layer was saturated with sodium chloride and then extracted twice with 2 ml of diethyl ether containing 20% *tert.*-butanol. The pooled ethereal extracts were methylated by bubbling diazomethane, which was generated according to the micro-scale procedure of Schlenk and Gellerman [23], through this solution until a yellow tinge became visible. After standing at room temperature for >2 min, the solvents were evaporated to dryness at 80°C under a stream of dry air. The residue was dissolved in 0.1 ml of ethyl acetate and 0.5–1 μl of this solution was injected into the GC–FPD system. The derivatization process is summarized in Fig. 1.

2.4. Gas chromatography

GC analysis was carried out with a Shimadzu 14A gas chromatograph equipped with a flame photometric detector (P-filter). A DB-17 fused-silica capillary column (15 m $\times$ 0.53 mm I.D., 1.0 μm film thickness) (J&W, Folsom, CA, USA) was used. The operating conditions were as follows: column temperature, programmed from 170 to 270°C at 10°C/min; injection and detector temperature, 280°C; and nitrogen flow-rate, 10 ml/min. The peak heights of herbicides and the I.S. were measured and the peak-height ratios against the I.S. were calculated.

2.5. Gas chromatography–mass spectrometry (GC–MS)

A Hewlett-Packard Model 5890A gas chromatograph was operated in conjunction with a VG Analytical Model 70-SE mass spectrometer and a VG-11-250J mass data system. A OV-1 fused-silica capillary column (25 m $\times$ 0.25 mm I.D., 0.25 μm film thickness) (Quadrex, New Haven, CT, USA) was used. The operating conditions were as follows: column temperature, programmed from 100 to 220°C at 8°C/min; injection temperature, 230°C; ion-source temperature, 230°C; ionizing voltage, 40 eV; helium flow-rate, 1 ml/min; and splitting ratio, 50:1.

3. Results and discussion

We had previously developed a GC method for the determination of GLYP and AMPA as their N-isoBOC methyl ester derivatives [22]. In preliminary tests, it was found that the tripeptide-type herbicide bialaphos could be derivatized and easily eluted from the GC column as its N-isoPOC methyl ester derivative rather than the N-isoBOC methyl ester derivative. Therefore, we tried the simultaneous determination of a series of the phosphorus-containing amino acid-type

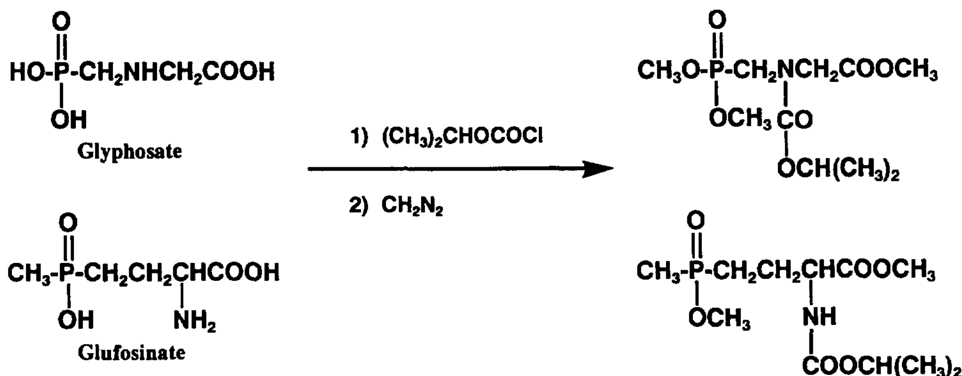

Fig. 1. Derivatization of glyphosate and glufosinate.

herbicides containing GLUF and bialaphos as their N-isoPOC methyl ester derivatives. However, the analytical reproducibility and sensitivity of bialaphos were poor in comparison with those for other herbicides. Therefore, in this work we investigated the simultaneous determination of AMPA, GLYP and GLUF.

These herbicides could be conveniently converted into their N-isoPOC methyl ester derivatives by essentially the same procedure as previously [22]. The derivatization process of GLYP and GLUF is shown in Fig. 1. The isopropoxycarbonylation of the amino function of these compounds was accomplished within 10 s in an aqueous alkaline medium by mixing with a vortex mixer at room temperature. Although the N-isoPOC derivatives of AMPA, GLYP and GLUF were not extracted into diethyl ether, the addition of *tert.*-butanol to the diethyl ether facilitated the extraction. The recoveries of N-isoPOC AMPA, GLYP and GLUF by extraction twice with diethyl ether containing 20% *tert.*-butanol were 98, 98 and 95%, respectively.

Subsequent methylation with diazomethane could be successfully carried out in this solvent. The mean deviatization of GLYP through the procedure established above was 94.2% ($n = 4$) by comparison with the reference derivative (>99% pure as judged by GC), which was prepared in essentially the same manner as the analytical derivatization procedure. The derivative preparation could be performed within 10 min.

The structures of the derivatives of herbicides were confirmed by GC–MS analysis. As shown in Fig. 2, a molecular ion peak (M^+) was observed for AMPA and GLYP but not for GLUF. Prominent fragment ion peaks at $M^+ - 59$ [$(CH_3)_2CHO$ or $COOCH_3$], $M^+ - 87$ [$(CH_3)_2CHOCO$], *m/z* 109 [$PO(OCH_3)_2$], *m/z* 93 [$PO(OCH_3)CH_3$], *m/z* 59 and *m/z* 43 [$(CH_3)_2CH$] were observed and these peaks were useful for structure elucidation. These derivatives were stable at room temperature, and no decomposition was observed during GC analysis.

As shown in Fig. 3A, AMPA, GLYP and GLUF were eluted at ca. 3.5, 5.8 and 8.1 min, respectively. Each compound was completely separated within 10 min and provided an excellent FPD response. The minimum detectable amounts of AMPA, GLYP and GLUF to give a signal three times the noise under our instrumental conditions were ca. 8, 12 and 20 pg injected, respectively. ABP was chosen as an I.S. because it was well separated from the herbicides investigated. In order to test the linearity of the calibration graphs, various amounts of these compounds ranging from 5 to 200 ng were converted, and aliquots representing 0.05–2 ng of each compound were injected. In each instance, a linear relationship was obtained, and the regression lines for AMPA, GLYP and GLUF were $y = 0.019x - 0.006$ ($r = 0.9982$, $n = 18$), $y = 0.013x - 0.021$ ($r = 0.9992$, $n = 18$) and $y = 0.006x + 0.006$ ($r = 0.9992$, $n = 18$), respectively, where y is the peak-height ratio against the I.S. and x is the amount (ng) of each compound.

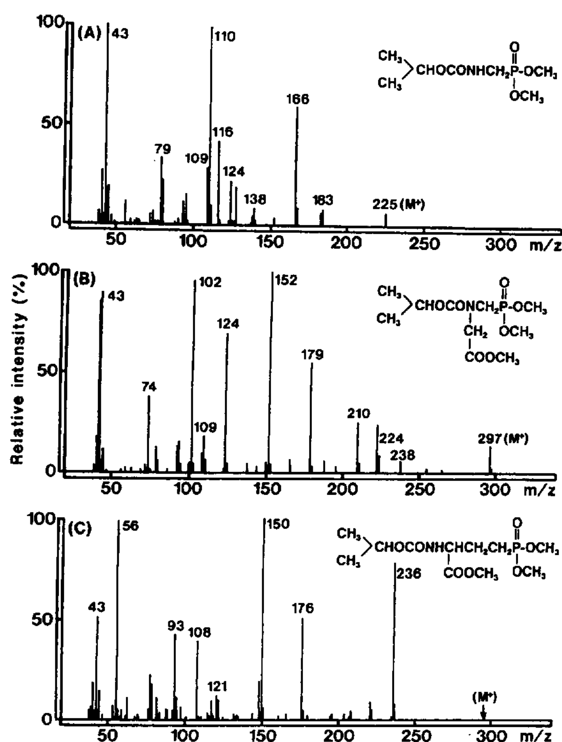

Fig. 2. Mass spectra of N-isopropoxycarbonyl methyl ester derivatives of (A) aminomethylphosphonic acid, (B) glyphosate and (C) glufosinate.

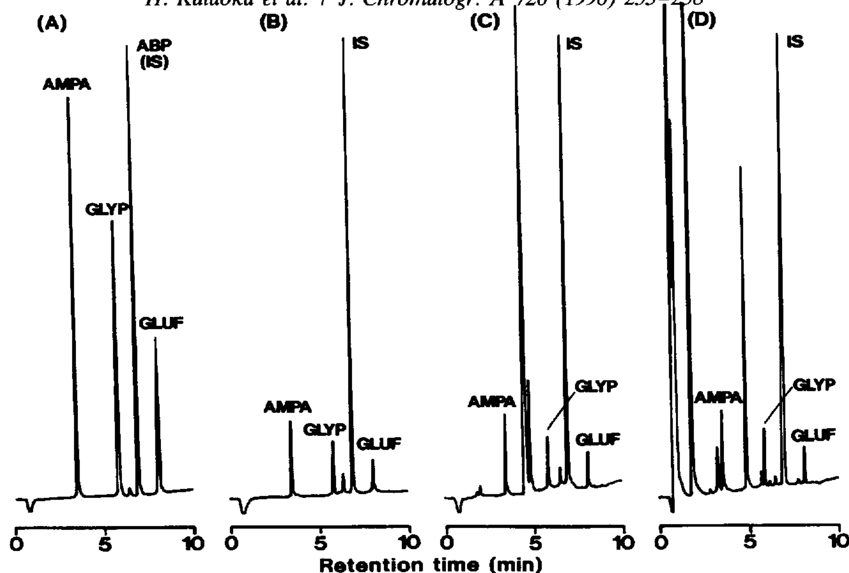

Fig. 3. Gas chromatograms obtained from standard solution and fortified samples. (A) Standard containing 50 ng of each compound; (B) river water fortified at 0.01 μg/ml; (C) clay loam soil fortified at 0.1 μg/g; (D) carrot fortified at 0.1 μg/g. GC conditions are given under Experimental. Peaks: AMPA = aminomethylphosphonic acid; GLYP = glyphosate; ABP = 4-aminobutylphosphonic acid (I.S.), GLUF = glufosinate.

In order to demonstrate the applicability of the method to environmental and food samples, AMPA, GLYP and GLUF in river water, soil, vegetables and crops were analysed. These compounds were extracted from soil samples under alkaline conditions as described previously [22,24]. On the other hand, these compounds in carrots could be extracted with distilled water as described previously [9,19]. The method developed could be applied directly to river water and the above extracts of soil and carrot samples without a preliminary clean-up procedure such as ion-exchange column chromatography. Fig. 3B–D show the chromatograms obtained from river water, clay loam soil and carrots fortified at 0.01, 0.1 and 1 μg/ml (or μg/g), respectively. Although the peak corresponding to 2-aminoethylphosphonic acid (retention time ca. 4.4 min) and an unknown peak (retention time ca. 4.8 min) were observed between AMPA and GLYP

Table 1
Recoveries of herbicides added to various samples

Sample	AMPA		GLYP		GLUF	
	Added[a]	Recovery[b] (%)	Added[a]	Recovery[b] (%)	Added[a]	Recovery[b] (%)
River water	0.01	104 ± 3[a]	0.01	104 ± 1	0.01	100 ± 0.3
	0.1	100 ± 3	0.1	102 ± 3	0.1	99 ± 3
Clay loam soil	0.1	99 ± 3	0.1	91 ± 5	0.1	91 ± 7
	1	101 ± 6	1	99 ± 3	1	100 ± 2
Carrot	0.1	98 ± 5	0.1	106 ± 7	0.1	94 ± 4
	1	94 ± 3	1	93 ± 2	1	96 ± 2

[a] Units μg/ml for river water and μg/g for clay loam soil and carrot.
[b] Mean ± S.D. ($n = 4$).

in soil and carrot samples, each compound could be detected without any interference from co-existing substances in all samples.

As shown in Table 1, the overall recoveries of AMPA, GLYP and GLUF added to several environmental and food samples were 91–106% and the relative standard deviations were 0.3–7.7% ($n = 4$). The quantitation limits of AMPA, GLYP and GLUF in river water samples, at a signal-to-noise ratio of 3, were 0.8, 1.2 and 2.0 ng/ml, respectively, and those in soil and carrot samples were 8, 12 and 20 ng/g, respectively. Several unfortified samples such as river waters, soils, cabbage and rice were analysed and these herbicides could not be detected in any of the samples.

4. Conclusion

Convenient and reliable methods for the simultaneous determination of phosphorus-containing amino acid-type herbicides in river water, soil and carrot samples were established. In comparison with previous methods, sample preparation and derivatization can be accomplished with simplicity and rapidity. The method is selective and sensitive, and various samples can be analysed directly without pretreatment except for extraction and without any interference from other co-existing substances. We believe that this method will provide a useful tool for environmental monitoring and residue analysis in foodstuffs.

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